Observations on the Protozoa Parasitic in the Hind Gut of Archotermopsis wroughtoni Desn.

Part I.—Ditrichomonas (Trichomonas) termitis, Imms.

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With Plates 31, 32,and 33, and 3 Text-figures.

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1. Introduction.

In a recent paper Dr. Imms (12)[1] has described some of the species of Protozoa parasitic in the hind gut of an Indian termite—Archotermopsis wroughtoni Desn. As many of these insects have been living in Dr. Imms' laboratory at Manchester, I had the opportunity, a few years ago, of examining their Protozoa, which showed a number of features of great interest and importance. Early in 1918 I decided to make a thorough investigation of these parasites, and Dr. Imms kindly placed at my disposal the remainder of his material. I therefore express to him my sincere thanks for thus rendering possible my research.

The object of the present paper is to record as fully as possible the life-history of one of the Protozoa. I hope to publish from time to time results of my observations upon the other species already described by Imms.

The flagellate forming the subject of this paper is one of the commonest species of Protozoa found in the termites ; it occurs in all casts in great numbers, and offers an excellent opportunity for work on its method of division.

The Trichomonads have been objects of research by many naturalists, but up to the present no species has been recorded from termites[2]; also, as will be seen later, the species I have investigated is in many respects totally different from any hitherto described.

Imms has already pointed out that the animal differs from other Trichomonads in the possession of only two anterior flagella, and this fact makes it desirable, though not essential, to place it in a new genus. As the genus Tetratricho-monas has been established for those forms possessing four

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[1] Although the above paper is still in the press, Dr. Imms has kindly given me permission to publish the results of my work.

[2] While this paper was in the hands of the printer I was able to read Grassi's latest publication on termite protozoa (' Mem. R. Accad. Lincei,' ser. 5, vol. xii, 1917). In this paper he describes a Trichomonad, which, however, differs considerably from D. termitis.

anterior flagella, but otherwise like Trichomonas, and the genus Pentatrichomonas for those possessing five anterior flagella, I have decided to create the new genus Ditrichomonas for the animal under discussion.

2. METHODS.

The animals were examined alive in 0·75 per cent. NaCl, but even when the utmost precautions were employed in the preparation of the slides, it was found impossible to keep the flagellates alive for more than about two hours. On occasion albumen in salt solution was used as a medium, but, though possessing the advantage of slowing the animal's movements, it caused rapid death, preceded by degeneration changes, thus rendering the use of this medium unsatisfactory.

From time to time I have endeavoured to obtain a pure culture of these parasites, but with no success. The ordinary bacteriological media were tried, as was also the culture media used by me in my experiments on Entamœba histolytica (5). Finally, I used an extract obtained from the wood in which the termites were living, and also a medium prepared by teasing up in salt solution the abdomen of a termite, but in all cases the results were completely negative.

Stained preparations were made by squeezing the contents of the hind gut of a termite out on to a clean slide; thin films were then prepared and the slides immediately placed in the fixing fluid. It is important that this should be done as rapidly as possible, as shrinkage effects are very soon produced after the Protozoa have left their host.

As fixing fluids I have used Bouin's picro-formol-acetic solution and Schaudinn's sublimate-alcohol mixture. Both these mixtures gave excellent results, especially when used at a temperature of about 56° C. The former, however, is disadvantageous in that the picric acid takes a long time to wash out before staining can be commenced. Equally good results were obtained by the use of Schaudinn's mixture as modified by Dobell and Jepps (8) as a result of their work on

Entamœba histolytica cysts. These bodies are very susceptible to shrinkage, and a fluid which obviates this is obviously one of great service to protozoologists. Such a fluid is prepared as follows : Saturated corrosive sublimate in water, two parts ; absolute alcohol, one part ; glacial acetic acid, 4 to 5 per cent.

For staining, Heidenhain's iron-hæmatoxylin is probably the best for the details of nuclear division, though Dobell's iron-hæmatein, described by him in 1914 (7), also gives fine results ; especially is it useful for work on the flagella and axostyles, which are better coloured by this method than by any other I have tried.

From time to time I have made use of alcoholic safranin, thionin, Grenacher's carmine, and Mayer's hæmalum, but have not obtained as good effects with any of these as with those mentioned above. Methyl green and Schneider's aceto-carmine have proved useful in those cases where permanent preparations were not essential.

Most of my slides have been made by one of the methods described above, but for the demonstration of the parabasal body I employed Flemming's strong fixing solution, omitting the acetic acid, as described by Gatenby (11).

3. General Considerations as to the Living Animal.

(a) Morphology and Movement.

Ditrichomonas termitis is a large flagellate measuring on the average $50\,\mu \times 22\,\mu$. There is, however, much variation in size, as occurs in most species of Protozoa ; indeed, Wenyon (27) states that in Trichomonas intestinalis he observed forms ranging from $3\,\mu$–$20\,\mu$.

Imms gives the size of Ditrichomonas termitis as $30\,\mu$–$88\,\mu$ in length by $13\,\mu$–$57\,\mu$ broad, with an average of $64\,\mu \times 38\,\mu$. These figures are undoubtedly too high, and this is due to the fact that Imms has included in his measurements both dividing and non-dividing animals, the former of which are of course much larger than the latter.

At the anterior end of the animal there is found a large cytostome, situated a little to one side of the middle line. Close to this structure there is the point of origin of the flagella, which are three in number. Two of these are directed anteriorly and are of equal length, while the third runs down one side of the body to form the edge of the undulating membrane, which is supported by a firm, rod-like structure characteristic of Trichomonas. The flagellum, however, becomes free at the posterior extremity of the body.

The body is not sharply differentiated into ectoplasm and endoplasm, but appears homogeneous throughout; the protoplasm is usually granular, though small vacuoles are occasionally seen, but in no case is there evidence of a contractile vacuole. In the interior of the body there are numerous food-particles, chiefly consisting of what appear to be fragments of wood, many of which are so large it is difficult to realise how the animal could engulf them. I have, however, been unable to detect any method of feeding other than by the cytostome.

As Imms has observed, the shape of the animal is not constant, but when progressing forwards it has a characteristic appearance, the anterior end broad and rounded, with this width maintained for about two-thirds of the body-length, the posterior third is, however, much more slender and in some cases almost tapering. Further, this slender region is capable of independent movement so that it is possible to speak of it as a " tail " (Text-fig. 1, a). The undulating membrane runs from the anterior end of the animal down to the end of this " tail " and the axostyle is seen projecting from it (Text-fig. 1, a, b). This shape is, however, not the only one found, for when the animal is at rest—or at any rate not moving forward—an entirely different appearance is seen. The " tail " portion of the body disappears and the animal assumes an almost round shape. The posterior end of the undulating membrane is now no longer separated from the anterior by the whole length of the animal, but is more closely applied to it (Text-fig. 1, c and e, and Pl. 31, fig. 1).

An intermediate condition between the two extremes is seen in Text-fig. 1, d. These changes in shape are brought about by contraction, and it is often possible, when examining these animals alive, to see this cycle of changes taking place with great rapidity in the same animal. Text-fig. 1 was drawn freehand from a living preparation; it represents the

TEXT-FIG. 1.
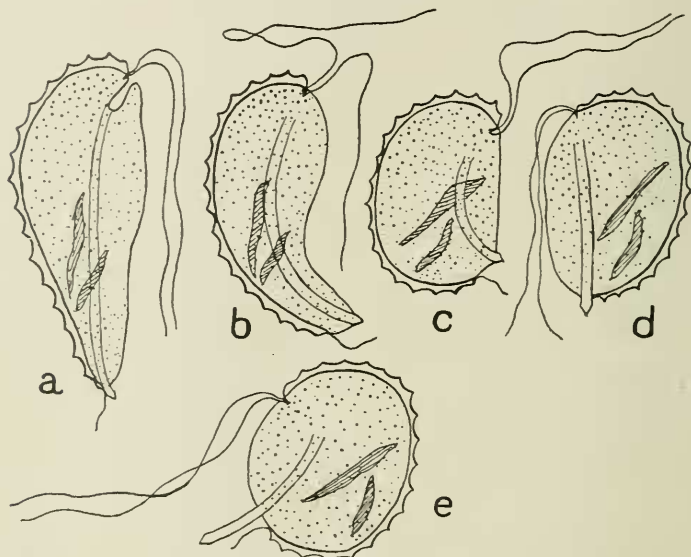
Freehand drawing of the form changes observed in a living Ditri-chomonas termitis during a period of five minutes.

successive movements of one of these flagellates after it had come to rest, the whole of the changes occurring in a period of five minutes.

I have spoken above of the animals being at rest; this, however, is only a relative term, for though they may not be undergoing translation, yet the free flagella and undulating membrane are quite active; indeed, the membrane appears to undergo its rhythmical changes throughout the whole of the animal's life. Minchin's suggestion (21, p. 57) that when the animal is perfectly still, "the function of the membrane is

probably to cause currents in the fluid surrounding the body, and to change and renew the liquid bathing the body surface " appears to me well founded.

Kuczynski (**19**, p. 163), has described the formation of pseudopodia in Trichomonas augusta observed alive in a bouillon culture.   He says : " 9h54′ Vorn rechts entsteht (ventral) ein Pseudopodium, um rasch nach hinten zugleiten, 3–4mal hinterein ander, dadurch wird der Eindruck der undulierenden Bewegungen hervorgerufen.   Dabei macht die Membran 90 Schläge in der Minute bei geringer Windungszahl. Sie ist klar erkennbar.   Nach allen Seiten werden spitze Pseudopodien ausgestreckt und wieder eingezogen.

" Blitzschnell mehrere zugleich.   Die Pseudopodien wandern den Körper entlang.   Die Geisseln arbeiten kaum.   Das Tier bewegt sich ganz langsam ein wenig von der Stelle (kriecht).   Es ist freigekommen, nimmt seine alte Gestalt wieder annähernd auf, schwimmt fort, zunächst anregelmassig bewegt.   Der Achsenstab steht weiter aus dem Körper als vorher.   (Dauer des Geschilderten drei Minuten.)

" 9h57′ : Es zeigt wieder am Hinterende den Haken.

" 10h7′ : Das Tier hat eine ganz normale Form ; es schwimmt, wobei sich 90 Umdrehungen in der Minute fest-stellen lassen.   Etwa ebenso oft schlagen die Greisseln."

Appearances such as the above I have sometimes seen in Ditrichomonas, when examined in media containing albumen.   In 0·75 per cent. NaCl, however, the animals show no trace of pseudopodial formation.   I do not doubt, therefore, but that this is an abnormal feature of the life-history, and that it is due to the effect of artificial media ; this conclusion finds support in that the forms which developed pseudopodia were in a feeble condition, and if watched for a short time became moribund.   Buscalione and Comes record the same fact (**4** a).

(B) Axostyle.

The axostyle, which is such a constant organ of the Trichomonas body, arises from the anterior end in front of

the nucleus, and probably has its origin in the blepharoplast, described on p. 563. It appears as a hyaline structure running throughout the body length (Pl. 31, fig. 9). At the posterior end it reaches the outer surface, which it pierces, and is continued for a short distance, finally terminating in a sharp point (Pl. 31, figs. 2, 3, 6, and Text-fig. 2, p. 564).

Kuczynski (**19**, **19a**) considers that it is composed of two threads running parallel to each other; this I think to be a mistake, but apart from this error his description could apply very aptly to the axostyle of D. termitis.

On p. 143 of his paper (**19**) he says : " Er besteht (i. e. the axostyle) bei sämtlichen bisher untersuchten Trichomonaden aus zwei Fribrillen, welche vom Basalkorper ventral vorn Kern und diesem dicht angeschmiegt, aber dorsal vom Cytostom, wenn dieses gut augebildet ist, die eine über der anderen entlang ziehen und nach geraden oder mehr oder minder gekrummtem Verlauf die Körper—peripherie erreichen."

Arranged in a linear series down the middle of the axostyle there are small, deeply-staining granules (Pl. 31, figs. 4, 6, 10) I have been unable to determine the significance of these, but they are of constant occurrence, and have been described in other species by previous workers. It is possible that they are the result of metabolic activity.

When preparations are made by fixing with the fluids mentioned on p. 580, it is found that clustered round the anterior portion of the axostyle there are another set of cytoplasmic bodies. These are small, deeply-staining, rod-like structures (Pl. 31, fig. 8). From their general appearance and from the fact that they are well seen only after treatment by the methods recently described by Gatenby (**11**) I believe that they probably represent mitochondria. It may be mentioned at once that similar bodies are sometimes found scattered through the cytoplasm in an irregular manner (Pl. 31, fig. 5), but in the majority of cases these mitochondria are aggregated round the axostyle in the way described. I am able to offer no suggestion as to the reason for this, but possibly they corre-

spond to the "Zona chromidiale" described by Grassi (11a) as encircling the anterior end of the axostyle (Mestolo) in Jœnia.

On observing a large number of living animals it is noticed that the amount of axostyle projecting from the body is very variable. Sometimes only the extreme point is seen (Pl. 31, fig. 7), while at others a relatively large part protrudes (Pl. 31, fig. 3). This also is observed when a single animal is studied through the form changes previously described. It appears as though, when the body is contracted into the rounded condition, the axostyle is too long to be accommodated, and is therefore pushed out. It is not, however, absolutely rigid, for when living animals are examined in those forms which are long and tapering in shape, the axostyle is seen running through the body in a straight line, but in the rounded forms it is flexed at some point in its course, as is seen by reference to Text-fig. 1.

I have not observed the axostyle used as an organ of attachment as Wenyon (27) described in T. intestinalis, but disputed by Dobell (6) from his observations on T. batrachorium. This structure, however, exhibits in D. termitis a very peculiar movement when the animal is at rest. The portion projecting from the body undergoes a slow, lateral jerking movement, which is confined to the region outside the body, as may be readily seen by the flexion occurring at the place where the axostyle becomes free.

Kofoid and Swezy (18) state that the axostyle exhibits "a vigorous lashing from side to side, sometimes constant, sometimes intermittent." Such a vigorous motion I have not observed, but it is evident, I think, that the authors have seen a phenomenon similar to the one recorded by myself.

4. Description of Stained Preparations.

(A) Anterior Granules and Nucleus.

In stained preparations there is seen at the anterior end of the body two distinct granules. From one—the blepharoplast —there arise the two free, anteriorly directed flagella of length

about equal to that of the body. From the second granule, which Imms does not seem to have noticed, the chromatic base of the undulating membrane and the flagellar border

TEXT-FIG. 2.
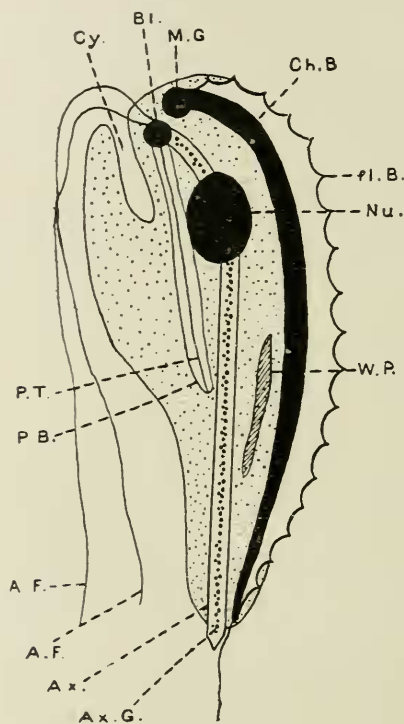
Semi-diagrammatic freehand drawing of a non-dividing Ditri-chomonas termitis. *Bl.* Blepharoplast. *A. F.* Anterior free flagella. *Cy.* Cytostome. *M. G.* Membrane-granule. *fl. B.* Flagellar border of undulating membrane. *Ch. B.* Chromatic base of membrane. *Ax.* Axostyle. *Ax. G.* Axostyle granule. *P. B.* Parabasal body. *P. T.* Parabasal thread. *Nu.* Nucleus. *W. P.* Ingested food-particle of wood.

of the same take origin. This granule I propose to term the "membrane granule," as it takes no part in nuclear division (Pl. 31, figs. 4, 5, 6, and Text-fig. 2).

A little posterior to the granules there is found the nucleus,

oval in shape and surrounded by a distinct membrane, the chromatin completely filling it as an homogeneous mass. The size is 6–8 μ.

There is no connection with the blepharoplast by a rhizoplast as described by Kofoid and Swezy in T. augusta. Imms' statement that such a structure is to be found in D. termitis is incorrect, and the probable explanation of the mistake will be found on p. 567.

(B) Parabasal Body.

Arising from the blepharoplast there is a long, deeply-staining body, which is undoubtedly that named as parabasal by other authors (Text-fig. 2). This is a constant constituent of the animal's body, and when preparations are treated in a suitable manner (see p. 580) it stands out most prominently. The parabasal extends for about two-thirds of the distance down the body, and has an almost straight course, though occasionally it shows a few undulations (Pl 31, fig. 5). There is, however, no coiling round the axostyle as occurs in Devescovina striata described by Foa (**10**).

Apparently the parabasal is composed of an homogeneous plasma not enclosed in a definite membrane. When the stain, however, is greatly extracted from it there is seen running down the middle a thread, which arises at the anterior end, and is attached to the blepharoplast (Pl. 31, fig. 6, and Text-fig. 2). This is evidently the parabasal thread described by Janicki (**13**) in other flagellates.

In all the preparations fixed by the method described on p. 580 the parabasal body is constant in size and position, exhibiting none of the fluctuations recorded by other workers, and especially by Kofoid in his recent paper (**18a**), in which he puts forward the view that this structure is a reservoir of kinetic energy, which supposition he largely supports on the variations seen in the parabasal bodies of the same species of animal.

I should mention that in " Schaudinn "-fixed material such variations are frequently encountered in D. termitis.

5. DIVISION.

(A) Anterior Granules and Nucleus.

The first indication of division is that the anterior flagella become four in number (Pl. 31, fig. 7). I have examined very many preparations in the hope that it would be possible to determine with certainty whether this doubling was brought about by division of the pre-existing flagella or by the growth of two new ones from the blepharoplast. I think that the latter is the correct view, for it is possible to find forms in which the flagella are of unequal length—two equal long ones and two equal short ones, which I conclude are the new ones in process of growth. The basal granule or blepharoplast from which they arise is not seen as a double structure until the four flagella have attained an equal length. When this occurs, however, it divides, and two equal granules are observed, from each of which two flagella spring (Pl. 31, fig. 8).

Simultaneously with the division of the blepharoplast— occasionally a little later—the membrane granule increases in size, and ultimately divides (Pl. 31, figs. 8, 9, 10). From the second granule so formed the new chromatic base grows out until it has attained a length equal to that of the old structure. There can be no doubt that this is the mode of origin, as all growth stages have been repeatedly seen, and are shown in Pl. 31, figs. 11, 12, 14, and Pl. 32, fig. 13.

During the growth of the chromatic base, the new undulating membrane is produced by the growth from the granule of a flagellar border, until a complete structure, similar in every way to the old one, is formed (Pl. 31, figs. 14, 15). I can find no evidence in D. termitis that the daughter-membrane is formed by division from the existing one as described by Dobell (6) and by Kofoid and Swezy (17); rather my results substantiate Wenyon's assumption (27) as regards the origin of the structure in T. intestinalis.

I would point out also that both the flagellar border and the chromatic base are produced from the same granule, and

not, as Wenyon states is the case, where the base arises from one granule, and the border from the same granule as do the anterior flagella.

When the animal has reached this condition its size is greatly increased, ranging from 60–80 μ. The nucleus has also grown, so that it is about 8 μ in diameter. At this stage the chromatin has contracted away from the nuclear membrane into a small mass, and lying between this and the membrane there are numerous small granules (Pl. 31, fig. 15, and Pl. 32, fig. 16), representing, I believe, the intra-nuclear cloud described by Kofoid and Swezy.

During these changes one of the blepharoplasts—probably the original one—divides once again, and the resulting body, the centriole, migrates towards the nuclear membrane, retaining its connection with the granule by means of a short thread—the rhizoplast (Pl. 31, fig. 11 A). Probably it was this stage in the life-history which led Imms to the conclusion referred to on p. 565—that the nucleus was attached to the blepharoplast by a rhizoplast.

The centriole now in its turn divides, and the two so produced are joined together by a solid strand of deeply-staining material—the paradesmose (Pl. 31, figs. 11A, 12, 14).[1]

At a later stage the second centriole acquires a connection with the other basal granule by the development of a new rhizoplast, which is of secondary growth.

As a result of these divisions a trapezoid figure is produced, very characteristic of the nuclear division (Pl. 32, figs. 13, 17, and Text-fig. 3, p. 568).

Finally the paradesmose assumes a position outside the nuclear membrane. This extra-nuclear position is retained throughout all stages; there is no evidence for its ever becoming intra-nuclear.

In the meantime the nucleus undergoes changes. Its

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[1] I prefer to follow the nomenclature used by Kofoid and Swezy (17), for the reasons given by these authors. The paradesmose refers therefore to the similar structure designated as " centrodesmose " by other authors.

original homogeneous character is lost and it becomes resolved
into a number of small granules (Pls. 31, 32, figs. 8, 14, 17) ;
occasionally there is a well-marked karyosome (Pl. 33, fig. 32),
but this a variable constituent of the nucleus and disappears
before division takes place. Kuczynski (**19**) has described a
similar body in the species of Trichomonas he has inves-
tigated.

TEXT-FIG. 3.
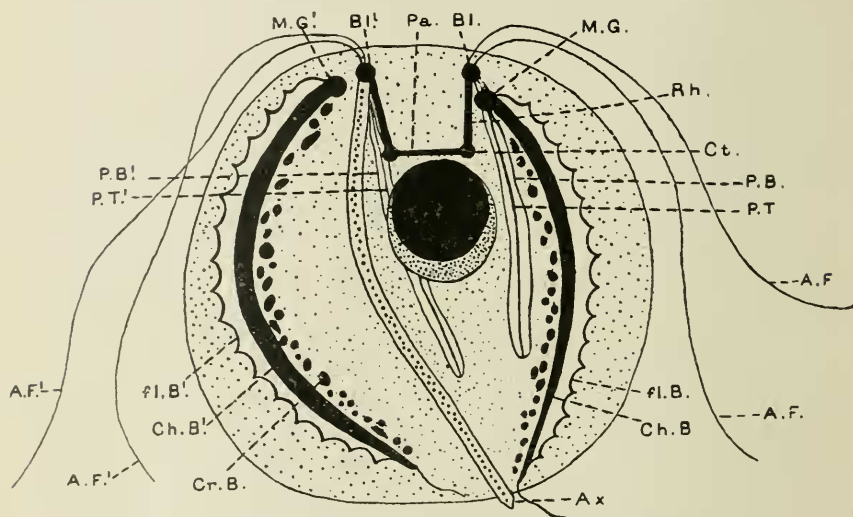
Semi-diagrammatic freehand drawing of a dividing Dicho-
monas termitis. A double set of organella has been formed,
together with the paradesmose and centrioles. *Bl.* Blepharo-
plast. *Bl'.* Daughter-blepharoplast. *A. F.* Anterior flagella.
A. F'. New anterior flagella. *M. G.* Membrane-granule. *M. G'.*
New membrane-granule. *fl. B.* Flagellar border of membrane.
fl. B'. New flagellar border of membrane. *Ch. B.* Chromatic
base of membrane. *Ch. B'.* Chromatic base of daughter-
membrane. *Cr. B.* Chromatinic blocks (probably metabolic
granules). *Ax.* Axostyle *P. B.* and *P. T.* Parabasal body and
thread. *P. B'.* and *P. T'.* Daughter-parabasal body and thread.
Rh. Rhizoplast. *Ct.* Centriole. *Pa.* Paradesmose.

From the nucleus there is now extruded a part of its
chromatin as a cloud of granules—the extra-nuclear cloud
of Kofoid and Swezy (Pl. 32, fig. 13). I have not been able

to determine this point with certainty, but I think it highly probable that the intra-nuclear cloud furnishes this extruded chromatin. It is certain that after the development of the extra-nuclear cloud the intra-nuclear one disappears.

Numerous observers have described in the cytoplasm of the body small refractile bodies which stain intensely by chromatin stains, and have been termed chromidial blocks, cytoplasmic bodies, etc. These seem to be a very constant feature of most Trichomonads, and I have found them in D. termitis at certain stages of the life-history. In this animal they vary in size from $1-2\cdot2\,\mu$ and are usually round in shape. In suitable preparations there is an indication that they are not homogeneous in character, but are formed of a central vesicle surrounded by a lightly-staining zone (Pl. 32, fig. 18).

Whereas, however, in many species of Trichomonas, such as T. gallinarum, described by Martin and Robertson (**20**), these bodies are a constant constituent of the animal, in D. termitis they are rarely seen in the non-dividing stage of the life-history. Division forms with a double set of organella and with the nucleus in the granular condition invariably contain them. In some cases they are few in number, but in others they are numerous and almost fill the body (Pl. 32, fig. 18); usually they are arranged in a series along the chromatic base of the membrane (Pls. 32, 33, figs. 23, 24, 25, 27, 30). When found in the vegetative forms they are present only in those which are the products of a recent division (Pl. 31, fig. 9); apparently as these young animals grow the granules are gradually absorbed so that the mature forms entirely lack them. Rarely have I seen them in a non-dividing form such as is shown in Pl. 31, fig. 5.

There is no evidence that these bodies undergo division during the reproductive stages of the flagellates.

It seems probable that they represent products of metabolic activity—a view which would to a certain extent account for their abundance in dividing animals.

To return to the account of the nuclear changes which occur during division. After the formation of the extra-

nuclear cloud the granules inside the nuclear membrane, which are indefinite in number, aggregate to form spherical masses—the so-called chromosomes—whose number does not seem to be definite; in most cases there appear to be six of equal size (Pl. 32, fig. 13). I have, however, found animals in which the number is between four and seven, and in these cases there is usually an inequality of size (Pl. 32, figs. 18, 19, 20).

Previous investigators, with one or two exceptions, record a constant number. Thus Wenyon states that there are six chromosomes in T. intestinalis; Kuczynski (**19**) finds eight in T. augusta; while Kofoid and Swezy state that in T. augusta, T. muris, Tetratrichomonas prowazeki and Eutrichomonas serpentis there are invariably five chromosomes. The discrepancy in the results of these authors, in some cases working on the same species, suggests that in many trichomonads the chromosome number is not constant. A discussion, however, of this point is to be found in Kuczynski's recent paper (**19a**), which unfortunately only came into my hands at the moment when the present paper was going to the press.

Soon after their formation the "chromosomes" elongate, forming short rods (Pl. 32, fig. 21) which split longitudinally (Pl. 32, fig. 22). The two halves of each body now draw apart (Pl. 32, fig. 23), so that two masses, each of approximately the same amount of chromatin, result (Pl. 32, figs. 24, 25).

It has been recorded by Kuczynski, Martin and Robertson that the chromosomes first pair and then split longitudinally before passing to each pole of the nucleus. I can find no evidence in D. termitis for this preliminary pairing, nor for any form of reducing division.

The whole of these processes occur within the nuclear membrane and there are no spindle-fibres produced. Hand in hand, however, with the nuclear changes the paradesmose elongates so that the centrioles occupy positions at each end of the nucleus, and during the whole period of separation the

chromosomes remain aggregated in loose bunches, with their apices very close to the separating centrioles (Pl. 32, fig. 23). The impression is forcibly given that the centrioles act as dividing centres for the chromosomes. It must, however, be clearly understood that the paradesmose and centrioles are during the entire cycle outside the nuclear membrane, and that there is no trace of any connection, in the shape of fibres, between the two structures. In the next and last stage of division the paradesmose and nucleus elongate still more until finally a constriction appears in the membrane of the latter (Pl. 32, fig. 26), so that ultimately two daughter-nuclei are produced (Pl. 33, fig. 27).

Further elongation of the paradesmose results in the separation of the nuclei so that a condition is reached like that seen in Pl. 33, fig. 28. Nuclear reconstruction occurs by a reversal of the process described above.

As the centrioles are connected by rhizoplasts with the basal granules, these, together with the flagella and membrane-granules, migrate from one another.

When the daughter-nuclei are separated by practically the whole width of the body the paradesmose disappears (Pl. 33, figs. 29, 30). There is no evidence that the centrioles remain in connection with the nucleus—indeed, I do not believe that this is the case—but that they share the same fate as the paradesmose.

6. Division of the Axostyle.

There is much discrepancy between the accounts given of the formation of the axostyles in Flagellates, and a short discussion of this will be found on p. 574.

Unfortunately I have not been able to work out as completely as is desirable the method of origin of the new structures in D. termitis. The axostyle does not stain at all well, and it is only occasionally that it can be traced throughout its entire length. In a few of my preparations, however, treated, as far as I am aware, in exactly the same way as others, the axostyles appear fairly plainly. These

preparations demonstrate that the old structure does not disappear during nuclear division, for it can be seen running throughout the whole body (Pl. 32, figs. 23, 24). In a few cases also there are indications that at the close of the telophase the old axostyle divides longitudinally, beginning at the anterior end (Plate 33, fig. 31). I believe that the process is similar to that described by Kofoid and Swezy in T. augusta, but unfortunately I cannot furnish such con-clusive evidence as they adduce for this conclusion.

One thing, however, is certain—that the new axostyles are not derived from the paradesmose, for on one or two occasions there was seen the paradesmose, connecting the separated nuclei together with the daughter-axostyles running through the body. Such a condition is seen in Pl. 33, fig. 28, which is, I think, sufficient evidence for concluding that there is no connection between the paradesmose and the axostyles.

7. Division of the Parabasal Body.

The parabasal body becomes duplicated directly after the blepharoplast and membrane-granule have divided, and before the formation of the paradesmose. Unfortunately I have been unable to follow the stages in this process, but I think that the new body is developed by the division of the old one. In all the many animals I have examined the two parabasals are seen lying side by side, each attached to its basal granule by the parabasal thread (Pls. 31, 33, figs. 15, 32 and Text-fig. 3). If, now, the daughter-structure arises by growth, one would expect to find the various intermediate stages as in Stephanonympha, and as one sees in the development of the undulating membrane. This I have never observed, however; in all the animals possessing two para-basals they are always of approximately the same size and appearance.

At the close of nuclear division the parabasals move in company with the basal granules away from each other, so that appearances such as are seen in Pl. 33, figs. 29, 30, are produced. In this way each daughter-animal possesses a

parabasal body and thread connecting it with the blepharoplast. The sequence of stages in the division of the animal is commonly that just given, but small variations in the course may occur. Thus the membrane-granule may divide before the blepharoplast (Pl 31, figs. 9, 10), or the nucleus may become resolved into chromatin granules before the development of the paradesmose (Pl. 31, fig. 8) ; these variations, however, are of little importance, and in no way affect the general course of events.

8. Sexual Process and Cyst-Formation.

In agreement with the majority of workers I have been unable to find any evidence for the presence of a sexual process.

Also there appears to be no cyst-formation—which is contrary to the general experience. However, after a careful search through many slides, involving the examination of the contents of numerous termites, I am forced to the conclusion that such a stage does not occur in the life-history of D. termitis. I may add that Imms, during the course of his research on these flagellates, was unable to find any cysts. Of course it is possible that during certain seasons of the year the animals may pass into the cystic stage ; but these conditions evidently do not obtain in the laboratory, as the termites have now been examined, at short intervals, throughout the whole year. Again, it is possible that the temperature conditions under which the animals live ·in India are such as to induce cyst-formation, and that these conditions are not realised in this country. Owing, however, to the habit which termites have of eating the fæces of their companions, often doing so directly it has left the anus, infection from animal to animal can easily occur without the aid of cysts ; this involves the assumption that the parasites are capable of withstanding the digestive juices of their hosts. That this is possible is indicated by the fact that I have often found the mid-gut of the termites heavily infected by all the species of Protozoa resident in the hind-gut.

This absence of cyst-formation is the experience of other workers on termite parasites, and Grassi (**11a**), in order to account for this in J œ n i a and M e s o j œ n i a, has put forward the same suggestion as the one outlined above. On p. 739 he says: "Probabilmente la soppressione dell' incistamento e for'anche quella della fecondazione sono rapportabili al costume or ricordato dei Termiti di mangiar la feccia dei propre compagni all'atto della emissione."

9. Multiple Fission.

Kofoid and Swezy (**18**), in their most recent communication to the American Academy, of which I have only been able to read a summary (**16**), state that in T. a u g u s t a, T. m u r i s, T e t r a t r i c h o m o n a s p r o w a z e k i and E u t r i c h o m o n a s s e r p e n t e s multiple fission occurs. According to these authors eight nuclei are produced with the accompanying flagella apparatus before the plasma divides. Eventually, however, eight daughter-animals are formed, which do not separate immediately.

Since this phenomenon was described in four closely related species, it seemed to me possible that it would also be found in D. t e r m i t i s. After a careful search, however, I have failed to find any trace of such an occurrence.

A review and criticism of the above work is given by Kuczynski (**19a**).

10. General Considerations.

(a) Axostyle.

The axostyle has been the subject of discussion as regards its origin and connection with the nuclear division, and also as to its relationship in the various orders of Flagellates. From the various descriptions given of this organ it appears that there are, broadly speaking, three modes of origin. In the first the daughter-axostyles are formed from the para-desmose (centrodesmose) which persists after the close of nuclear division, as described by Prowazek (**23**), Dobell (**6**), and Janicki (**14**). In the second method the new axostyles

grow out from basal granules and have no connection with
the paradesmose, which disappears at the end of division of
the nucleus. This method is decribed by Kuczynski (**19, 19a**).
The supporters of these two methods are in agreement that
the old axostyle disappears and plays no part in the formation
of the new ones. Although Kuczynski is, I think, incorrect
in his statement that the new axostyles arise by the growth of
threads from basal granules, yet he has made the interesting
and important observation that they are developed before the
disappearance of the paradesmose. As will be remembered,
I was able to show the same thing occurring during the
reproduction of D. termitis. The third type of origin of
the axostyles was first described by Wenyon (**27**) in T.
intestinalis, and has recently been re-described by Kofcid
and Swezy (**17**). Here the old structures divide longi-
tudinally at the close of the telophase, thus giving origin to
the new axostyles. This is the method which I believe to
have found in D. termitis.

There seems to me, however, to be little doubt that some
axostyles do arise from the paradesmose as Dobell stated.
In Lophomonas this is most certainly the case, as may be
verified by anyone who will study the various division-stages
in these animals; further, the account given by Dobell for T.
ranarum and by other students of different species of flagel-
lates leaves little room for doubt that this method obtains.

On the other hand, the results obtained by Kofoid and Swezy
completely negative this view, as does Kuczynski's statement
of finding the paradesmose and axostyles present at the same
time. Such a condition as I found in D. termitis and figure
in Pl. 33, fig. 28, of this paper is to me conclusive.

It is, therefore, evident that the axostyles of various flagel-
lates have different origins and are not homologous with one
another, but rather analogous. Surely no one would assert
that the bundle of axial fibres, running down the middle of
the body of Calonympha and Stephanonympha, as
described by Janicki (**15**), and formed by the union of an
axial fibre from each nuclear complex, is homologous with the

Trichomonas axostyles, yet they are probably similar in function with one another.

As Jollos (**16**) truly remarks in his abstract of the work of Kofoid and Swezy, it is necessary to obtain a clear idea of the relationship of the axostyles in the various groups of Polymastiginidæ, and until this is done it is hopeless to homologise them with other structures as so many people have attempted to do, some regarding them as cytoplasmic flagella, and others likening them to the thread running down the middle of the spermatozoon tail.

My observations, and those of others mentioned above, make it impossible to support certain statements made by Janicki (**14**) in 1912. On p. 99 of his paper he says: "Was ich als allgemeines Resultat der vorliegenden Zusammenstellung besonders hervorheben möchte, ist der Umstand, dass das Auftreten der extra-nucleären Spindel wahrend der Kernteilung sich bei den Gattungen Konstatieren lässt, welche mit einem Achsenstab resp. dessen Homologa versehen sind."

And again on p. 100: "Das der Achsenstab, sei es in seiner Grundlage (Jœnia, Trichomonaden, Devescovina), sei es überhaupt in seiner Gesamtheit (Calonympha, Stephanonympha) auf die persistierende extra nucleäre Spindel (Zentralspindel) zuruckzufuhren ist, kann heute als gesicherte Erkenntniss gelten, die für Flagellaten, wie schon gesagt, zum erstenmal durch Grassi unter Mitwirkung von A. Foa an Jœnia begründet wurde."

Finally I may add as additional proof of the opinions which I have stated above, that in other parasitic flagellates of the Termites, an account of which I hope to publish later, the axostyle cannot arise from an extra-nuclear spindle, because such a structure does not occur in these forms.

(B) Undulating Membrane, Blepharoplast, and Nuclear Division.

So much has been written in the past regarding the first two of these structures that it is unnecessary for me to do

more than indicate the most important points which have arisen from my research.

A striking feature of the life-history of D. termitis is the perfect independence of the blepharoplast and the membrane-granule, though in Devescovina and Parajœnia the trailing flagellum has an origin distinct from that of the anteriorly directed flagella.

In many species of Trichomonas, however, the free flagella and the undulating membrane arise from a single body—the blepharoplast—such as in T. batrachorum and T. gallinarum, and there is no trace of differentiation into separate bodies.   Prowazek (24) concluded that this terminal basal granule was in reality tripartite, and composed of three granules closely associated.   Parisi (22) finds in T. prowazeki and T. orthopterorum a double blepharoplast, but he does not state whether the undulating membrane springs from one of these or not.   Some unpublished observations of my own on this latter species, however, lead me to conclude that the arrangement is similar to that of D. termitis.

Wenyon (26) found in T. intestinalis two distinct granules lying close together, from one of which the anterior flagella and the flagellate border of the membrane arose, and from the other the chromatic base took origin.   Benson (4), however, describes in T. vaginalis the same arrangement as I have given in this paper.

It appears as though we have before us in the species of Trichomonas a gradual elaboration of the blepharoplast. Starting from a primitive condition where each flagellum has its own basal granule, the next stage is found in those forms in which two or three granules have fused to form a complex such as we have in D. termitis, or as in T. vaginalis, if three granules fuse, leaving the membrane-granule free.   In the final condition this also is absorbed into the complex, so that a single multipartite body is produced from which all the locomotor apparatus arises, and this may play a part in nuclear division, thus forming a true blepharoplast.

A further point of interest which has arisen out of the work

is the demonstration that in division the daughter-membrane is produced by independent growth and not by division of the existing membrane. This method has been described by most of the earlier workers, but Dobell (6) asserted that in T. batrachorum the flagellar border was formed by splitting of the old one, and this view has been revived recently by Kofoid and Swezy (17). As I have already said, there is no evidence whatsoever to be found in D. termitis for such an opinion.

Turning now to consider nuclear division, one is struck by its independence of the bodies governing the flagella. In many flagellates the blepharoplast acts as division centre, so that it has been suggested that this granule and the metazoan centrosome are homologous structures. Dobell (7a), how-ever, in a recent communication on Oxnerella maritima, a Heliozoan, contests this view. On p. 535 of his paper he says : " The centroplast of the Heliozoa is thus closely com-parable with the blepharoplast of the Mastigophora—an organ permanently subserving a skeletal function to the organs of locomotion (the flagella), and in some forms assuming the office of centrosome at division, in others playing no part in this process (as in some trichomonads and in Copromonas respectively, as I have shown in two earlier papers [1909 and 1908]). To say that either the centroplast or the blepharo-plast is the homologue of the metazoan centrosome and to apply the same term to all these structures appears to me, therefore, inadvisable. . . . In the language of the older morphology, I would say that the centroplast and the centro-some may be analogous, but are not homologous, organs." With this view I am in complete accord.

In many Trichomonads there appear to be no division-centres developed. Recently, however, Kofoid and Swezy (17) state that in the forms they investigated the blepharoplasts migrate to the poles of the nuclear membrane and there divide to form centrosomes. On p. 318 of their paper they state that " the two blepharoplasts have migrated to the two poles of the pointed ellipsoidal nucleus, and each has divided

into a centrosome at the apex of the spindle and the adjacent
basal granule to which the flagella are attached. In some
instances the division of the blepharoplasts is not apparent.
. . . Connecting the two blepharoplasts as they migrate
to the polar position is a heavy chromatic thread, which lies
outside the nuclear membrane. This we name the para-
desmose, though in origin it may seem to be homologous
with the central spindle of the metazoan mitotic figure." This
statement is a little vague, and leaves one in doubt as to
whether the paradesmose connects the centrosomes or the
basal granules. It serves to show, however, that there is little
demarcation between the two bodies. Now in D. termitis
they are quite distinct and act independently, the basal
granules retaining their position at the anterior end of the
body, while the centrioles derived from them migrate to
the nuclear poles. Thus it appears that in this animal the
complex blepharoplast of other forms is here easily resolved
during reproduction into its constituent parts.

Finally I would emphasise the fact that the paradesmose
is a transitory structure. That it has no connection with the
axostyles I have already shown, but I wish it to be understood
also that the centrioles disappear at the close of nuclear
division, and new ones are produced at the next division from
the blepharoplasts. I have no wish to enter into a discussion
regarding the centriole of the Protozoan nucleus, for this
controversy has already become somewhat wearisome, but
I might point out that my observations supply a further
proof—if such is necessary—of the untenability of the now
famous phrase of Hartmann and Chagas that " Das allgemeine
Vorhandsein von Zentralorganem im Caryosom aller Proto-
zoen kann jetzt als eine wohlbegrundete wissenschaftliche
Tatsache gelten."

(c) The Parabasal Body.

On p. 565 I have said that in every specimen of D. ter-
mitis there is found a well-developed parabasal body.
In the previous literature on Trichomonas this body has

been mentioned, but, with the possible exception of T. batra-
chorum, as recorded by Alexeieff (1) and Janicki (15), it has
only been found sporadically. Because of this, Kuczynski,
in his earlier paper (19), considered that it was of transitory
appearance. In his recent communication (19a), however, he
states that such a body is a constant character of the newly-
described T. mirabilis. He says, however, that during
division the structure passes over entire to one of the daughter-
animals, and is formed " de novo " in the other. This is, of
course, in direct opposition to the conclusion at which I have
arrived.

I think that the non-appearance of the parabasal in so
many cases is due to the method of preparing the slides.
In all my preparations fixed with sublimate or Bouin's fluid
the parabasal was only occasionally seen, and then very
indistinctly—so much so that for a long time I was in doubt
as to what the faintly-stained, badly-defined body was which
I recorded in my notes. When, however, I fixed material
with Fleming's solution, as modified by Gatenby (11)—that
is, by omitting the acetic acid—and then stained with iron-
hæmatoxylin, the body was most distinctly seen, and I at
once recognised that I was dealing with the parabasal body
described by other workers.

Subsequent work has shown that the same result is obtained
after fixation with osmic acid, 5 per cent. formol, or any
good fixative so long as sublimate and acetic acid are not
constituents.

I may mention here that I have found Fleming's solution,
modified as described above, an excellent fixative for Protozoa,
and would recommend it to protozoologists. In the past
workers have tended to use only one fixative, and that
Schaudinn's fluid, which, though excellent in many ways,
may lead to erroneous conclusions, as in D. termitis. I
by no means advocate that Gatenby's mixture should be
substituted for Schaudinn's, but that in an investigation
these and other good fixatives should be more extensively
employed, and so obviate as far as possible the errors which

arise through the different actions that fixing fluids exert upon the protoplasm of the cell. That these are often very great may be seen by reference to the papers recently published by Gatenby (**11**), where he demonstrates the existence of many "cell-inclusions" overlooked by previous observers who had been too conservative regarding their use of fixatives

For staining the parabasal any reliable Protozoan stain is suitable, including Delafield's hæmatoxylin, though this is contrary to Janicki's experience.

The structure of the parabasal in D. termitis is not remarkable in any way, though the presence of the parabasal thread is of interest, as it has not previously been described in Trichomonas. Such a connection with the blepharoplast, however, is recorded by Swezy (**25**) in Polymastix bufonis, and in other flagellates, such as Devescovina, Parajœnia and Stephanonympha. In division the parabasal seems to behave in three different ways among the various orders of Flagellates. Thus it may—

(A) Be destroyed in the mother animal and formed anew in each daughter-individual—Lophomonas.

(B) Divide and pass to the daughter-animals in connection with the division centres—Devescovina, Parajœnia.

(C) Remain undivided at one pole of the nuclear spindle and a new one grow from the other pole—Stephanonympha, T. mirabilis.

For the reasons given on p. 572 I consider that the body in D. termitis belongs to the second category.

It is of interest, however, that, owing to the independence of the centrioles and the basal granules, the parabasals remain attached to the latter, and have no connection with the division centres at the poles of the paradesmose, as occurs in the animals mentioned above.

Various suggestions have been made as to the significance of the parabasal—that it is of the nature of waste products, or that it is allied to the mitochondria (Janicki). For this latter view there is the support that the mitochondria and parabasal are affected by the various fixatives in the same

manner, but our knowledge of the parabasal is at present too small to warrant any definite statement. A great deal more detailed work is required before such discussions would be profitable.

It is necessary, however, to refer briefly to the latest suggestion of Kofoid and Swezy, who homologise the para-basal of other Flagellates with the chromatic base of the undulating membrane. This view is, I think, quite untenable. It has been shown that parabasals do occur in some species of Trichomonas, and though Kofoid and Swezy do not describe them in T. augusta, yet they were recorded in this species by Alexeieff in 1909 and by Kuczynski in 1914. It appears probable also that when research is made upon suitably prepared material the presence of these bodies will be found in most, if not all, the species of Trichomonas.

Such being the case, it is unlikely that the chromatic base represents the homologue of the parabasal of the Trichonym-phidæ, and that the body described in trichomonads as parabasal is an entirely new structure. Of course such a con-dition is possible, but until very definite proof is adduced for such an hypothesis it is legitimate to view it with the utmost suspicion. A further criticism of this view is given by Jollos (**16**). In the ' University of California Publications,' vol. xvi, 1916, Swezy has elaborated her views as to the nature of the parabasal, and Janicki in his last paper gives a full discussion of the subject. Also Kuczynski (**19b**) adduced reasons for believing that the parabasal and the kinetonucleus are homologous, which view is also held by Kofoid (**18a**). To these papers I would refer the reader who is desirous of obtaining full discussions of this subject.

11. Diagnosis of Ditrichomonas termitis.

I will conclude this paper by giving a brief diagnosis of Ditrichomonas termitis, which may be useful to future observers, and will at the same time render unnecessary a summary of the foregoing facts.

Ditrichomonas termitis.—Tetramitidean flagellate of

large size (average $55\,\mu \times 22\,\mu$).   At the anterior end there are two free flagella springing from a blepharoplast, and a posteriorly directed one forming the border of the undulating membrane and arising from a special granule.   Cytostome present.   From the anterior extremity of the body there arises an axostyle which runs through the whole body-length, emerging at the posterior end.   This structure shows a peculiar lateral movement of its free portion in the living animal.   An elongate parabasal body with a centrally situated thread springs from the blepharoplast.   The cytoplasm is granular, and in some animals filled with small, deeply-staining granules resembling mitochondria.   The nucleus is anteriorly placed, the chromatin completely filling the space bounded by a well-developed membrane.

The only method of reproduction observed is by simple division into two.   The blepharoplast divides, as does the membrane-granule from which the new undulating membrane grows.   By division of one blepharoplast a centriole is produced, which divides to form two.   These migrate apart, and between them a solid band of fibres—the paradesmose—is formed.   This becomes situated just outside the nuclear membrane and remains there throughout the division stages.   Nuclear division is by a simple mitosis without fibres.

The daughter-parabasal is probably produced by the longitudinal splitting of the existing one.   The old axostyle is not absorbed during the reproduction stages, but probably divides to form the daughter ones.   It is certain that the paradesmose plays no part in their origin.

Habitat.—Hind-gut of an Indian termite—Archotermopsis wroughtoni Desn.

Food.—Particles of wood and cellulose.

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DESCRIPTION OF PLATES 31 to 33.

Illustrating Mr. D. Ward Cutler's paper, " Observations on
the Protozoa Parasitic in the Hind-gut of Archotermopsis
wroughtoni Desn. : Part I.—Ditrichomonas termitis,
nov. gen., nov. spec."

[With the exception of figs. 1, 2, 3, all the figures have been drawn
from permanent preparations with the aid of a camera lucida and the
following optical apparatus: Zeiss apochromatic oil-immersion objective
2 mm. (N.A. 1·3) and compensating ocular 6. Critical illumination
was always employed. The magnification of all figures except figs. 1,
2, 3 is approximately 940 diameters; figs. 1, 2, 3 were drawn freehand
from living preparations. Figs. 5, 6, 8, 10, 15, 25, 29, 30, 31, 32, 33 are
from preparations fixed with Fleming's fluid, without acetic acid, as
described by Gatenby. The remaining figures are from preparations
fixed with either Schaudinn's fluid or the modification of it recommended
by Dobell and Jepps. Figs. 6, 7, 9, 15, 23, 29, 31 are from preparations
stained by Dobell's hæmatein stain, the remaining figures from prepara-
tions stained by Heidenhain's iron-hæmatoxylin. See Text-figs. 2 and
3 for reference to organella, etc]

PLATES 31, 32 and 33.

Figs. 1, 2, 3.—Living animals, examined in 0·75 per cent. NaCl,
showing the general body characters. Note the large amount of axostyle
projecting in fig. 3.

Fig. 4.—Normal non-dividing form. Parabasal body not visible.
Axostyle with centrally-arranged granules.

Fig. 5.—Non-dividing animal with deeply-stained parabasal and
scattered mitochondria. The distinction between blepharoplast and
membrane-granule is well seen.

Fig. 6.—Same as above, but less deeply-stained so as to show the
parabasal thread. Axostyle with the series of granules.

Fig. 7.—Animal in the first stage of division with four free flagella ;
blepharoplast still undivided.

Fig. 8.—Blepharoplast divided and also the membrane-granule.
Parabasal with thread. Mitochondria clustered round anterior end of
the axostyle.

Fig. 9.—Small form in which membrane-granule has divided before
the blepharoplast.

Fig. 10.—Same as above : axostyle arising from blepharoplast.

Fig. 11.—Animal with two membrane-granules, from one of which the new chromatic base is beginning to grow out.

Fig. 11A.—Centrioles have been formed from one of the basal granules.

Fig. 12.—Centrioles joined together by the paradesmose. Further growth of the chromatic base of the new undulating membrane. Nucleus breaking into granules.

Fig. 13.—Trapezoid figure produced from the centrioles, basal granules and rhizoplasts. Paradesmose outside the nuclear membrane. Beginning of growth of the flagellar border of undulating membrane. Nucleus with extra-nuclear cloud and the chromatin aggregated into six "chromosomes."

Fig. 14.—Formation of the paradesmose and second undulating membrane ; nucleus with intra-nuclear cloud.

Fig. 15.—Animal in which the organella are duplicated. Two parabasals seen. Nucleus with intra-nuclear cloud, and the beginning of extra-nuclear one.

Fig. 16.—New undulating membrane almost completed. Intra-nuclear cloud in nucleus.

Fig. 17.—Stage previous to fig. 13, with nucleus breaking into granules.

Figs. 18, 19, 20.—Forms in which the chromosomes are of various numbers. In fig. 18 the metabolic granules almost fill the body.

Fig. 21.—Chromosomes assuming the rod-like form.

Fig. 22.—Longitudinal splitting of the chromosomes.

Fig. 23.—Elongation of the paradesmose ; chromosomes separating into two bunches. Axostyle still present throughout the body.

Fig. 24.—Chromosomes completely separated into two masses inside the nuclear membrane. Metabolic granules along the chromatic bases. Complete axostyle still present.

Fig. 25.—Similar to above, but parabasal bodies seen.

Fig. 26.—Animal in which nuclear membrane is beginning to constrict.

Fig. 27.—Nucleus completely separated into two, which are in the granular condition.

Fig. 28.—Daughter-nuclei widely separated, but still connected by paradesmose. Note the two daughter-axostyles.

Fig. 29.—Paradesmose has disappeared. Two parabasals in connection with the blepharoplasts. Two axostyles present.

Fig. 30.—Similar to above, but without the axostyles visible. The nucleus is losing its granular condition.

Fig. 31.—Animal in which axostyle appears to be dividing.

Fig. 32.—Animal with stain extracted so as to show the parabasal threads. Mitochondria are visible; and the nucleus contains a karyosome.

Fig. 33.—Animal at the end of division: the two daughter-forms each possess an axostyle and parabasal body.